THE METABOLISM OF SERUM ALBUMIN IN VIRAL HEPATITIS

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1962

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A thesis presented to the faculty of the
Yale University School of Medicine
in partial fulfillment of the
requirements for the degree of
Doctor of Medicine
1962

Acknowledgment and thanks are extended to Dr. H.O. Conn for his constant encouragement and invaluable assistance in the formulation and completion of this work. Dr. D.L.Buchanan is also extended thanks for making available the facilities of the Radioisotope Unit of the West Haven Veterans Administration Hospital.

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Introduction:

Many different hepatic functions are deranged in viral hepatitis and albumin, which is synthesized in the liver, has been shown to undergo quantitative and qualitative changes in this disease state. Previous investigators have shown that there is a diminution of serum albumin levels in hepatitis.[10,16,23] and disturbances in the thymol turbidity and cephalin cholesterol flocculation reactions in hepatitis are dependant primarily on the quantitative and qualitative changes in the serum globulins and albumin.[20]

Despite the availability of the radioiodinated albumin technique introduced by Sterling[30], which is a reliable and relatively simple method for studying albumin metabolism, no investigation of albumin metabolism in viral hepatitis has been undertaken. The only observation bearing on this problem was made by Sterling in his initial report where he presented data about one patient with hepatitis in whom the half-life of albumin was greatly shortened.[31]

Since viral hepatitis is, in most instances, a self limited disease, it presents an ideal opportunity to study the acute effects of the disease on albumin metabolism and then, using each patient as his own control subject, to restudy these patients after recovery.

This study was undertaken to investigate albumin metabolism in acute viral hepatitis using the radioiodinated albumin technique.

Materials and Methods:

A. Clinical Material:

All patients studied had been admitted to the West Haven Veterans Administration Hospital or the Grace-New Haven Community Hospital. Subjects for the study fell into three groups:

1. Control Group: Control subjects consisted of normal medical students or patients with psychiatric disorders in normal physical health. They ranged in age from 23 to 49 years. None had albuminuria. The control subjects ate a normal diet and their weight was stable throughout the period of the study.

2. Cirrhosis Group: Each of the patients had clinically evident Laennec's Cirrhosis which was confirmed histologically. Each was in a stable phase of his disease. They ranged in age from 37 to 49 years and none had albuminuria.

3. Hepatitis Group: The subjects with viral hepatitis, who ranged in age from 28 to 64 years, were studied as soon as possible after their admission to the hospital. They were in various stages of the acute phase of their illness and had been admitted from 5 to 30 days following the onset of symptomatology. The degree of severity of the clinical disease varied considerably, and patients were classified as either severe, moderate, or mild hepatitis.* None had albuminuria.

* - Clinical summaries will be found in the appendix.

A control subject was studied simultaneously with each subject with hepatitis in order to control the technical aspects of the investigation. When possible, a cirrhotic patient was also studied at the same time.

Liver function tests were performed on each patient with hepatitis at weekly intervals and in the other groups at least once before and once at the conclusion of the study. Liver function studies, which consisted of direct and total serum bilirubin, bromsulfalein retention, cephalin flocculation, thymol turbidity and serum alkaline phosphatase, were performed according to methods previously described from this laboratory.[7] Quantitative total protein and albumin determinations were made at the onset of the study and at weekly intervals thereafter to evaluate whether the subjects were in a steady state, i.e. albumin production was equal to albumin degradation. Filter paper electrophoresis was performed on 0.01 ml of serum using the Spinco hanging-strip method. The strips were stained for protein with bromphenol blue and quantitated with the Beckman Analytrol.[34]

All the subjects were given 15 drops of Lugol's solution on the day prior to and the day following the injection of the radioalbumin to minimize thyroid uptake of the I-131.

B. Methods:

1. Preparation of radioalbumin for injection: The radioalbumin mixture used for injection was prepared by adding 4.0 ml

of 25% untagged human serum albumin (American Red Cross) to a sterile bottle containing 26.0 ml of physiologic saline, thus creating a concentration of approximately 28.5 mg albumin per ml. The radioactive iodinated albumin used was a commercial preparation (Albumotope^{R}, Squibb Laboratories) containing 50-65 mg protein per ml with benzyl alcohol as a preservative. Approximately 300 microcuries of this preparation was introduced into the saline-albumin mixture after the removal of a sufficient amount of the solution so that the dosage bottle contained exactly 30.0 ml with 10 uc of radioactive iodinated albumin per ml. Each subject was given approximately 50 uc intravenously. The volume injected was measured for calculation of plasma volume.

2. Collection and counting of samples: Blood specimens were collected in Vacutainer^{R} tubes containing ethylene diaminetetracetate (EDTA) (Sequestrene^{R}) at 15 minutes, 24, 48 and 72 hours, and 5, 7, 10, 12, 14, and 17 days after injection of the radioalbumin. Some variation in the time of collection occurred in several subjects. The specimens were centrifuged and 1.0 ml aliquots of plasma were prepared in duplicate for counting in a well-type scintillation counter (Atomic Instrument Co. Well counter)*. Standards were prepared by diluting 0.10 ml of the dosage mixture to 500 ml of which 1.0 ml aliquots were counted. Three consecutive 24 hour urine collections were made beginning at the time of injection. After total urine volume was recorded, duplicate 3.0 ml aliquots were prepared.

*- modification of Sterling's technique which involved precipitation of albumin and counting the dry samples on planchettes

All blood and urine specimens for each subject were counted on the same day at the conclusion of the test period to eliminate the necessity of correcting for radioactive decay. All counts were finally expressed as counts/minute/ml.

3. Calculations: From these values, the plasma volume was calculated by the use of Formula A:

$$\text{Formula A: Plasma Volume} = \frac{\text{counts standard X dilution factor}}{\text{counts of 15 minute sample}}$$

which is an application of the simple dilution principle.

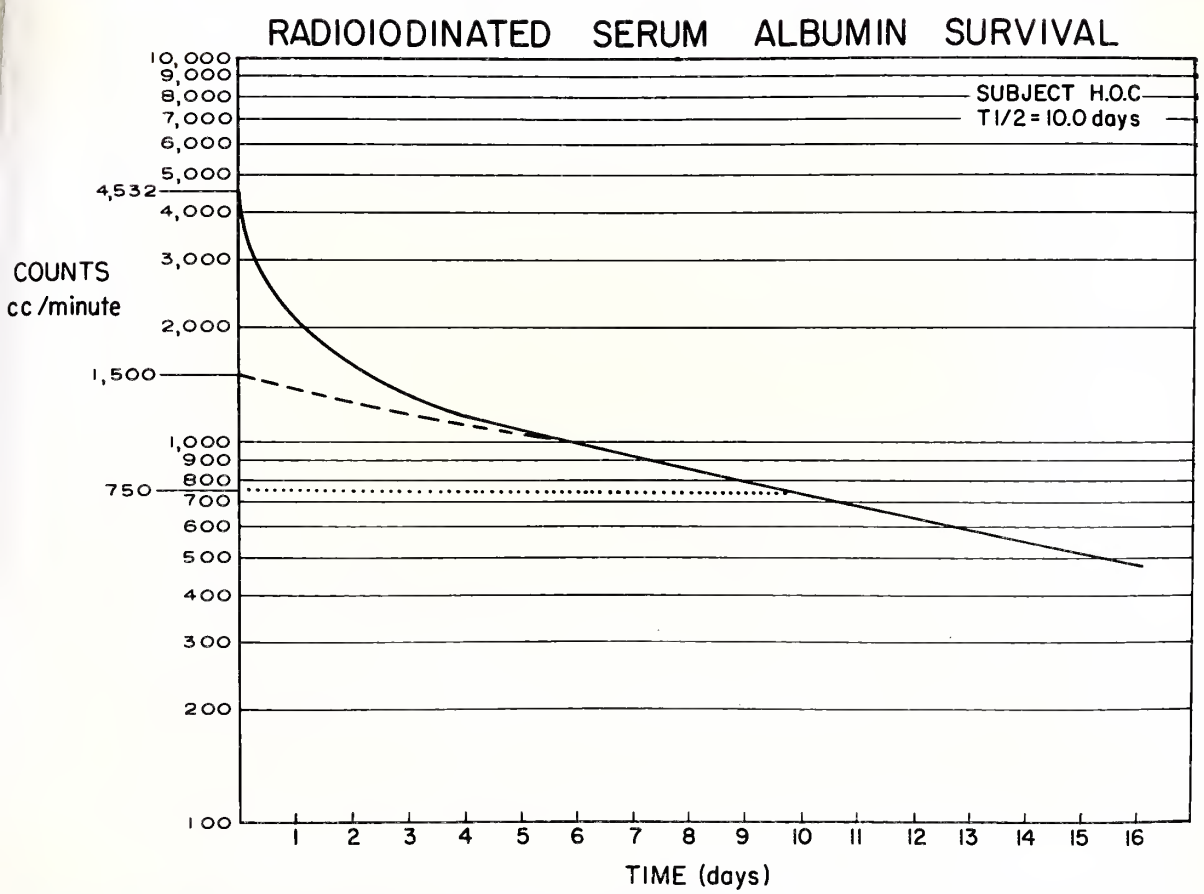
The radioactivity of the plasma samples (counts/minute/ml) was plotted as the ordinate against time (days) on semilogarithmic graph paper. The resulting curve consisted of two components, a rapid initial fall followed by a more gradual, linear decline. (Figure I) The rapid fall was interpreted as the period of intravascular-extravascular and intracellular-extracellular equilibrium,[22] while the linear portion represented the actual decay of the I-131 albumin. The extrapolation of the linear portion to zero time gave the value of albumin specific activity at the time of injection assuming complete distribution. By halving this value and determining its intercept on the graph, the half-life ($T\frac{1}{2}$) was determined. From this value the turnover rate was calculated by the use of Formula B:

$$\text{Formula B: turnover rate} = \frac{\text{natural log } 2}{T\frac{1}{2}} = \frac{0.693}{T\frac{1}{2}}$$

which is derived from the equation for exponential decay:

$$A = A_0 \, e^{-kt}$$

Figure I


RADIOIODINATED SERUM ALBUMIN SURVIVAL
SUBJECT H.O.C
T1/2 = 10.0 days
COUNTS
cc /minute
10,000
9,000
8,000
7,000
6,000
5,000
4,532
4,000
3,000
2,000
1,500
1,000
900
800
750
700
600
500
400
300
200
100
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
TIME (days)

where:

A = I-131 tagged albumin at any time
A_o = I-131 tagged albumin at zero time
t = time in days
k = turnover rate as fraction of the albumin turned over per day

The exchangable albumin pool was determined by the method of Sterling[30]:

Formula C: Exchangable Albumin Pool =

$$\frac{\text{Alb. specific activity at zero time (cts/ml)}}{\text{Quantitative albumin in plasma (mg\%)}} = \text{counts/mg albumin}$$

then by isotope dilution principle:

$$\frac{\text{counts injected}}{\text{Albumin spec. activity (counts/mg)}} = \text{grams albumin (E.A.P.)}$$

The circulating albumin was similarly determined, using the albumin specific activity at zero time assuming mixing with circulating albumin only, i.e. counts of 15 minute sample, as the numerator in Formula C.

The final results describing albumin turnover were expressed as functions of the exchangable pool, i.e. %/day; grams/day; grams/kg/day; and grams/1.73m^2/day.

The sample calculations for a normal control, H.O.C., are as follows: (See Figure I)

Standard: 2,813,809 counts/ml (undiluted)
15 minute sample: 4,531 counts/ml plasma
Albumin specific activity at zero time: 1500 counts/ml
Albumin half-life ($T\frac{1}{2}$): 10.0 days

$$\text{Formula A: Plasma Volume} = \frac{\text{counts of standard X dilution factor}}{\text{counts of 15 minute sample}}$$

$$\text{Plasma Volume} = \frac{2{,}813{,}809 \text{ X } 5 \text{ (ml injected)}}{4{,}531 \text{ counts/ml}} = 3104 \text{ ml}$$

Formula B: Turnover rate = $\frac{\text{natural log. 2}}{T\frac{1}{2}}$ =

Turnover rate = $\frac{0.693}{10.0}$ = 6.9 %/day

Total Protein: 6.75 gms%
Albumin: 3.5 gms%

Formula C: E.A.P. = $\frac{\text{Alb. spec. act. at zero time (counts/ml)}}{\text{Quantitative albumin (mg\%)}}$ =

= counts/mg albumin, then:

$\frac{\text{counts injected}}{\text{Alb. spec. act.}}$ = grams albumin (E.A.P.)

$\frac{1500 \text{ counts/ml}}{3.5 \text{ gms\%}}$ = 42.86 counts/ mg albumin

$\frac{2,813,809 \text{ X } 5}{42.86 \text{ mg}}$ = 327.9 grams = E.A.P.

For circulating albumin:

Formula D: $\frac{\text{counts of 15 minute sample}}{\text{Quantitative albumin}}$ = counts/mg albumin

$\frac{\text{counts injected}}{\text{alb. spec. act.}}$ = grams albumin (Circulating albumin)

$\frac{4,531 \text{ counts/ml}}{3.5 \text{ gms\%}}$ = 129.45 counts/mg/albumin

$\frac{2,813,809 \text{ counts X } 5 \text{ ml}}{129.45 \text{ counts/mg}}$ = 108.7 grams (Circulating albumin)

Results:

The mean half-life of albumin turnover in 9 normal subjects was 11.6± 1.4 days (Table I) with a mean turnover rate of 6.0± 0.7 %/day. In the three patients with cirrhosis, the albumin turnover ($T\frac{1}{2}$) was 13.3± 0.5 days with a turnover rate of 5.2± 0.4 %/day (Table II).

The mean albumin half-life in the 10 patients with hepatitis was 10.7± 2.5 days. The mean turnover rate was 6.8± 1.6 %/day (Table III). However, in this group there were included two patients who were treated with adrenocortical steroids during the course of their illness and their half-life was much shorter than the rest of the group, averaging 7.7 days with a turnover rate of 9.0 %/day. Excluding these two patients, the remainder of the hepatitis group had a mean albumin half-life of 11.4± 2.2 days and a mean turnover rate of 6.3± 1.2 %/day.

Three patients with hepatitis were restudied after their recovery. It was found that during their illness the mean albumin $T\frac{1}{2}$ was 12.2± 3.3 days and the mean turnover rate 5.7± 1.3 %/day; after recovery, the mean half-life of albumin was 13.1 ± 1.4 days, the mean turnover rate being 5.4± 0.9 %/day (Table IV).

The mean value for the exchangable albumin pool (EAP), based on the electrophoretic value of serum albumin, was 355 ± 64 grams in the normal subjects, 345 ± 44 grams in the patients with cirrhosis and 326 ± 79 grams in the patients with hepatitis (Table V).

ALBUMIN TURNOVER RATE IN NORMAL SUBJECTS

Subject	Weight in Kg.	Surface Area in M^2	Albumin T 1/2 in Days	Albumin in gms/100ml.	Exchangeable Albumin Pool in gms.	Circulating Albumin in gms.	Albumin Turnover Rate			
							%/day	gms/day	gms/Kg/day	gms/1.73M^2/day
J. C.	78	1.96	13.6	3.2	367	173	5.1	18.7	.240	16.5
H.O.C.	87	2.04	10.0	3.5	328	109	6.9	22.6	.260	19.2
C.D.K.	66	1.82	9.8	3.8	264	167	7.0	18.5	.280	17.6
E.T.P.	82	1.96	12.4	4.2	390	250	5.6	21.8	.266	19.3
M. Z.	57	1.74	13.0	3.8	296	133	5.3	15.7	.275	15.5
J. H.	82	2.14	11.6	4.1	377	141	6.0	22.6	.276	18.2
D. M.	65	1.74	10.8	2.7	345	112	6.4	22.1	.340	21.9
J. P.	91	2.10	12.4	4.1	472	176	5.6	26.4	.290	21.8
J. D.	73	1.96	10.6	3.2	--	--	6.5	--	--	--
Mean			11.6	3.6	355	158	6.0	21.1	.278	18.8
Standard Deviation			±1.4	±0.5	±64	±45	±0.7	±3.3	±0.04	±2.3

TABLE II

ALBUMIN TURNOVER RATE IN PATIENTS WITH CIRRHOSIS

Subject	Weight in Kg.	Surface Area in M^2	Albumin T 1/2 in Days	Albumin in gms/100ml.	Exchangeable Albumin Pool in gms.	Circulating Albumin in gms.	Albumin Turnover Rate			
							%/day	gms/day	gms/Kg/day	gms/1.73M^2/day
C. C.	81	1.92	13.6	3.3	324	250	5.1	16.5	.204	14.9
J. J.	58	1.74	14.2	1.7	395	131	4.9	19.4	.334	19.2
J. B.	72	1.88	13.2	3.4	315	163	5.7	18.0	.248	16.5
Mean			13.3	2.8	345	181	5.2	18.3	.262	16.9
Standard Deviation			±0.5	±0.9	±44	±61	±0.4	±1.4	±0.06	±2.2

TABLE III

ALBUMIN TURNOVER RATE IN PATIENTS WITH HEPATITIS

Subject	Weight in Kg.	Surface Area in M^2	Albumin T 1/2 in Days	Albumin in gms/100ml.	Exchangeable Albumin Pool in gms.	Circulating Albumin in gms.	Albumin Turnover Rate			
							%/day	gms/day	gms/Kg/day	gms/1.73M^2/day
R. H.	71	1.96	13.0	4.7	457	229	5.3	24.2	.341	21.4
R. F.	60	1.78	15.0	3.6	334	184	4.6	15.4	.257	15.1
J. D.	64	1.54	9.6	3.6	337	96	7.2	24.3	.380	25.9
E. A.*	70	1.94	12.8	2.3	279	146	5.4	15.1	.216	13.5
E. B.	90	2.08	9.6	3.3	355	109	7.2	25.2	.280	21.0
E. M.	65	1.82	12.2	2.5	192	114	5.7	10.9	.168	10.3
R. M.	68	1.82	10.4	3.6	401	134	6.7	26.9	.396	25.6
W.E.B.	79	1.98	8.6	3.3	---	---	8.1	----	----	----
A.D.**	74	1.88	7.8	2.9	300	132	8.9	26.7	.361	24.5
W.B.**	108	2.24	7.6	3.4	254	80	9.1	23.1	.214	17.9
Mean			10.7	3.3	326	136	6.8	21.3	.279	19.5
Standard Deviation			±2.5	±0.7	±79	±46	±1.6	±5.9	±0.08	±5.6

* toxic hepatitis
** received adrenocortical hormones therapeutically during study

TABLE IV

ALBUMIN TURNOVER IN PATIENTS WITH HEPATITIS STUDIED AFTER RECOVERY

Subject	Weight in Kg.	Surface Area in M^2	Albumin T 1/2 in Days	Albumin in gms/100ml.	Exchangeable Albumin Pool in gms.	Circulating Albumin in gms.	Albumin Turnover Rate			
							%/day	gms/day	gms/Kg/day	gms/1.73M^2/day
A. During acute illness:										
J.D.	64	1.54	9.6	3.6	337	96	7.2	24.3	.380	25.9
R.F.	60	1.78	15.0	3.6	334	184	4.6	15.4	.257	15.1
R.H.	71	1.96	13.0	4.7	457	229	5.3	24.2	.341	21.4
Mean			12.2	4.0	336	170	5.7	21.3	.326	20.8
Standard Deviation			±3.3	±0.2	±37	±55	±1.3	±5.1	±0.02	±5.5
B. After recovery:										
J.D.	57	1.54	10.8	4.2	319	104	6.4	20.4	.358	22.9
R.F.	63	1.78	13.8	3.6	237	107	5.0	11.9	.189	11.6
R.H.	73	1.96	14.6	3.6	304	109	4.7	14.3	.196	12.7
Mean			13.1	3.8	287	107	5.4	15.5	.248	15.7
Standard Deviation			±1.4	±0.3	±44	±2.6	±0.9	±4.4	±0.10	±6.2

Table V

Group	EAP	Circ. Alb.	Ratio: mean circ. alb./ EAP
Normal Control	355	158	0.45
Cirrhotics	345	181	0.52
Hepatitis	326	136	0.42
Sterling[30] Normal	263	117	0.45

The actual turnover rate of albumin determined by multiplying the turnover rate by the exchangable albumin pool was expressed as grams/day, grams/kg/day, and grams/1.73m^2/day. Results are summarized in Table VI.

TABLE VI

SUMMARY OF ALBUMIN TURNOVER RATES IN ALL SUBJECTS

Group	Albumin Turnover Rate in gms/day	in gms/Kg/day	in gms/1.73M^2/day
Normal subjects (8)	21.1 ± 3.3	.278 ± .040	18.3 ± 2.3
Patients with cirrhosis (3)	18.3 ± 1.4	.262 ± .060	16.9 ± 2.2
Patients with hepatitis (9) (All)	21.3 ± 5.9	.279 ± .080	19.5 ± 5.6
Patients with hepatitis (2) treated with steroids	24.9	.288	21.2
Patients with hepatitis (7) excluding those treated with steroids	20.3 ± 6.4	.291 ± .080	19.0 ± 6.1
Patients with hepatitis (3)	21.3 ± 5.1	.326 ± .020	20.8 ± 5.5
Above 3 patients after recovery	15.5 ± 4.4	.248 ± .100	15.7 ± 6.2

Discussion:

A. Methods:

The validity of the radioiodinated albumin technique for the study of albumin metabolism depends primarily on two assumptions:

1. that all of the I-131 is protein bound and remains so throughout the lifetime of the albumin, and

2. that the I-131 labeled albumin is not physiologically altered by the radioiodination procedure and that it behaves in vivo like native protein.

The commercial preparation used in this study (Albumotope^{R}, Squibb Laboratories) is manufactured by introducing iodine into the tyrosine moiety of the albumin molecule, incorporating a maximum of one atom of iodine per molecule at optimal pH and under conditions necessary to prevent protein denaturation.[25] Unbound iodine is removed by an ion exchange method and comprises less than 2.0% of the total iodine content of the final preparation, and usually is less than 1.0%.[25] The integrity of the protein molecule and the stability of the radioactive label are tested by paper electrophoresis and bioassay in dogs.[25] Dialysis of similar preparations by Bloom[4] as well as precipitation techniques of comparable commercial products by the author[15] have shown that all of the radioactivity except for a negligible fraction is protein bound in the plasma. Furthermore, Sterling[30] and McFarlane[17] have presented data to indicate that I-131 remains bound to the albumin molecule throughout its lifetime.

Theoretic considerations which cast some doubt on this conclusion will be discussed below. Immunologic studies as well as observations in other species have indicated that tagged albumin closely resembles untreated protein in physiologic behavior.[30] The minute amount of albumin injected does not affect the metabolism to any appreciable degree.

Differences in the rate of disappearance of radioalbumin labeled with C-14 and S-35 on the one hand and I-131 on the other have been observed by several investigators.[1,2,9,21,24,33] who noted that the albumin half-life of radiocarbon or radiosulfur-labeled albumin was almost twice that of the radiiodinated compound, i.e. the turnover rate was half as rapid (Table VII). The conclusions were that iodinated albumin may not be metabolized normally in man. Goldsworthy felt that these discrepancies may have been caused by alterations in the I-131 labeled protein occurring during isolation, iodination and handling processes prior to its administration.[9] Since most of these studies compare endogenously labeled albumin with C-14 or S-35 labeled amino acids with exogenously produced iodinated albumin, it was postulated that perhaps the prolongation of the half-life with C-14 or S-35 is caused by a reutilization of the radioactive elements following albumin degradation. Another possible explanation suggests that the chemically prepared radioiodinated albumin was degraded abnormally rapidly. However, some investigators have observed that 85-95% of C-14 is excreted within 10 days after

TABLE VII

ALBUMIN HALF-LIVES FOR NORMAL SUBJECTS, I^{131}, C^{14}, S^{35} and N^{15}-LABELED ALBUMIN

Investigator	Radioisotope label of albumin		Albumin half-life in days	
Armstrong (1)	I^{131}		9.7	
Dixon (8)	I^{131}		13.1	
Margen and Tarver (21)	I^{131}*	S^{35}*	15.0	24.0
	I^{131}		10.1	
Masouredis (24)	I^{131}	C^{14}**	12.4	33.5
Volwiler (33)		S^{35}**		19.3
London (16)		N^{15}**		20.1
Kushner (14)	I^{131}		11.8	
Blahd (3)	I^{131}		12.9	
Sterling (30)	I^{131}		10.5	
Berson (2)	I^{131}		17.0	
Kyropoulos	I^{131}		11.6	

* Simultaneous labeling of albumin molecule with S^{35} and I^{131}

** Biosynthetically labeled albumin

administration and thus discount the probability of reincorporation of the C-14 atom into the newly synthesized albumin molecule.[24]

McFarlane has shown the iodination of the albumin molecule with 6 or more atoms of I-131 per molecule affects the protein's physiologic activity by abnormally shortening its half-life. Albumin labeled with 0.5 atoms of iodine per molecule behaved in a fashion similar to C-14 and S-35 labeled protein and presumably physiologically in vivo,[17] although the preparation of the albumin itself differed in other ways as well. Other investigators employing McFarlane's technique for radioiodination and C-14 and S-35 labeled albumin prepared in donors found no appreciable difference in the disappearance rates in rabbits and rats.[5,6]

Margen and Tarver, using human serum albumin doubly labeled with methionine S-35 and I-131, showed that the half-life as determined by S-35 activity was 22.5 days and that determined by I-131 was 10.5 days. These experiments led these authors to conclude: "... that I-131 was lost in part from the albumin by a mechanism that did not involve the rupture of peptide bonds because this would have resulted in ... the loss of the S-35 label."[21] Volwiler and co-workers have independantly confirmed these findings.[33] This discrepancy in half-lives, determined on the same albumin molecules by two different radioactive labels, is similar to the half-lives determined when I-131 or S-35 labeled albumin are adminstered seperately. One may conclude that deiodination in vivo in the absence of albumin degradation is responsible for the apparent difference in albumin half-

life as determined by these different techniques.

B. Results:

The degradation curve of albumin is indicated as the semilogarithmic function of plasma radioalbumin activity plotted against time. It consists of two components, a rapid initial fall followed by a more gradual, linear decline. The rapid fall is interpreted to represent the period of intravascular-extravascular and intra-cellular-extracellular equilibration, while the linear portion represents the actual decay of the radioalbumin.[21] The validity of decay curves is based upon the assumption that a steady state exists with respect to albumin, that is that albumin production is equal to albumin degradation. In patients with viral hepatitis, the existence of a steady state is uncertain. One would expect, at least transiently, disturbances in the normal equilibrium of albumin metabolism. In the subjects in this study, who represent a group of patients with hepatitis of average severity, there were no apparent deviations from the steady state. All of the patients were maintained on adequate diets in terms of proteins and calories and exhibited stable body weight and serum albumin levels (Table VIII).

Several investigators have noted a diminution of the concentration of serum albumin in viral hepatitis.[10,16] Martin noted that there was a depression of the albumin component with a corresponding rise in gamma globulins as determined by serial electrophoretic examinations of the serum proteins.[23] There seemed

TABLE VIII

EVALUATION OF THE STEADY STATE IN PATIENTS WITH INFECTIOUS HEPATITIS AS SHOWN BY SERUM ALBUMIN LEVELS AND BODY WEIGHT AT THE BEGINNING AND END OF THE STUDY

Subject	Serum total bilirubin levels in mgm%		Albumin gms/100ml		Weight in lbs.	
	Before	After	Before	After	Before	After
R. H.	3.5	1.3	4.3	4.3	156	158
R. F.	2.0	1.5	3.6	4.1	132	129
J. D.	24.0	3.5	3.7	3.6	138	126
E. A.	3.0	1.7	2.2	2.5	154	153
E. B.	11.9	1.7	3.2	3.3	196	196
E. M.	8.9	7.2	2.6	2.4	143	142
W.E.B.	20.1	3.3	3.2	3.4	172	166
R. M.	1.2	0.3	3.5	3.6	150	151
A. D.	33.2	29.3	3.0	2.9	162	156
W. B.	21.8	4.2	2.7	2.8	237	228

to be a maximal depression within the first 10 days from the onset of the symtpoms which recovered steadily thereafter. In addition, there was a disturbance of the alpha-2 and beta globulins, notably between the 14th and 30th days.

In addition to these relatively large quantitative changes, qualitative changes in albumin have been postulated. In attempting to elucidate the mechanisms of the thymol turbidity and cephalin cholesterol flocculation reactions, Maglagan and Bunn noted that although normal albumin inhibited the flocculations caused by beta and gamma globulin components, the albumin from some patients with hepatitis did not. [20] They assumed that qualitative changes caused by the hepatitis were responsible.

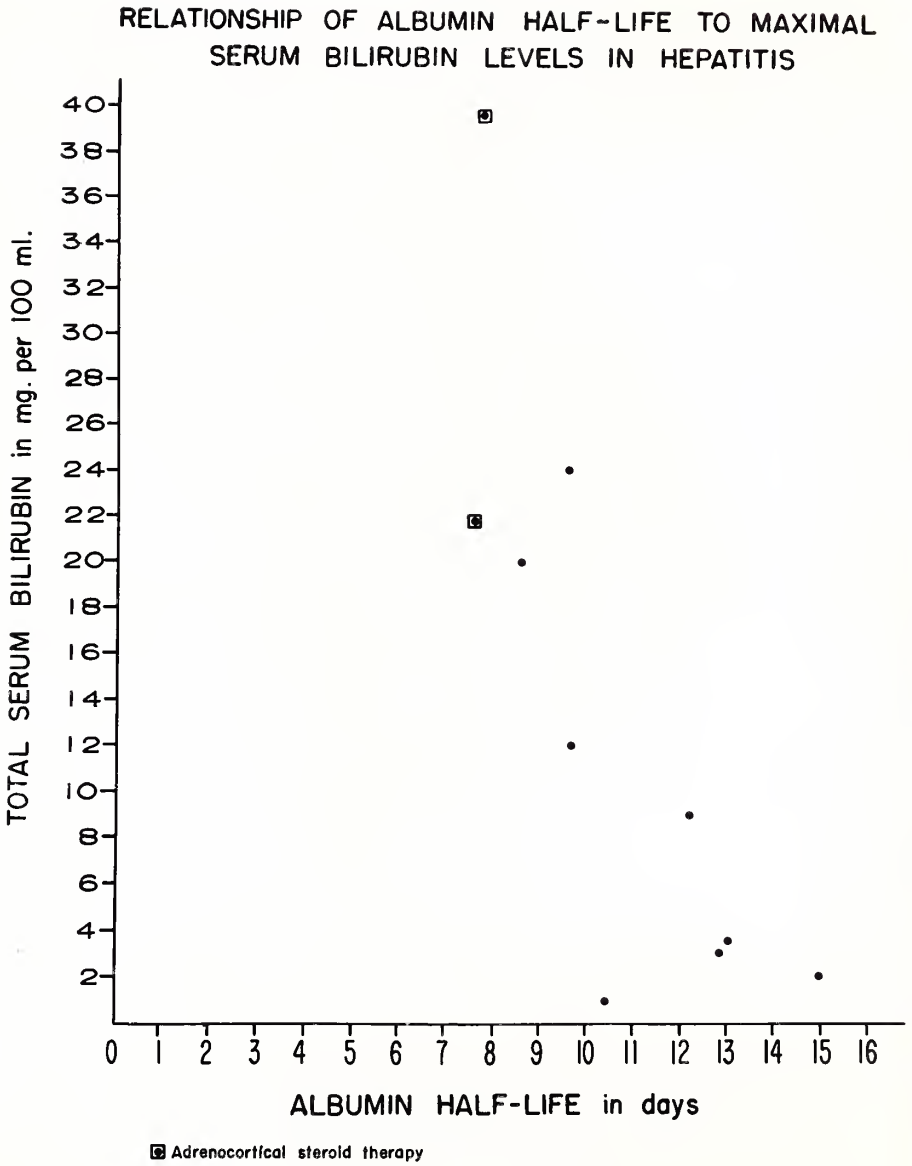
In view of the demonstration of both qualitative and quantitative alterations of serum albumin in hepatitis, it is logical to assume that the metabolism may be altered as well. Would such disturbances in albumin metabolism be reflected in changes in the survival time and turnover rate of the serum albumin? This was implied by Sterling who reported a single case of hepatitis with markedly deranged albumin metabolism.[31] His patient had a shortened albumin half-life of 7.4 days with a rapid turnover rate of 9.73 %/day. However, an unusually low exchangable albumin pool in this subject resulted in a near normal turnover rate, i.e. 14.1 grams/1.73m^2/day, the normal mean being 15.4 $\pm$ 2.0 grams/1.73m^2/day. This is in contrast to patients with low serum albumin levels, such as in cirrhosis, who will usually have relatively long half-lives and low albumin turnover rates, as was demonstrated by Sterling.[31]

The albumin half-life and turnover rate of the hepatitis patients in this study closely approximated those of the normal subjects. The three patients studied after recovery showed no significant change in their albumin turnover rates or half-lives from the values observed during their acute illness. The patients with cirrhosis exhibited prolonged albumin half-lives and slower turnover rates.

It was noted that the variation that occurred in the albumin half-life of the patients with hepatitis seemed to be systematically related to the severity of their illness. If one assumes that the serum bilirubin is an index of the severity of hepatic dysfunction, the data indicate that an inverse relation may exist between the maximal serum bilirubin level and the albumin half-life (Figure II). Further investigation is necessary to demonstrate such a correlation.

Two patients with viral hepatitis (A.D. and W.B.) had shortened half-lives, Indeed, their turnover rates were almost twice those of the other hepatitis patients. Both of them, because of the severe clinical manifestations of their disease, received adrenocortical steroids therapeutically. Both had low exchangable albumin pools, although the absolute albumin turnover rate was still somewhat accelerated. The shorter half-life is compatible with the increase in albumin turnover induced by steroids. Grossman et al. administered hydrocortisone and corticotropin to 10 normal subjects and demonstrated a marked increase in albumin degradation.[10] Rothschild and co-workers found that albumin degradation increased by a mean of 27% in 5 male patients after the administration of adrenocortical

Figure II


RELATIONSHIP OF ALBUMIN HALF-LIFE TO MAXIMAL SERUM BILIRUBIN LEVELS IN HEPATITIS
TOTAL SERUM BILIRUBIN in mg. per 100 ml.
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
ALBUMIN HALF-LIFE in days
Adrenocortical steroid therapy

hormones.[27] It is impossible to conclude whether the shortened half-life in my two patients is related to the severity of the hepatitis or the steroids.

The normal mean for exchangable albumin pool as determined in this study was 345 ± 78 grams. This was higher than that obtained by Sterling in his initial study[30] (mean for normals: 232 ± 48 grams), but more closely approximates the same author's later work[32] (314 ± 48 grams). The ratio of circulating albumin to the exchangable albumin pool, however, was the same (Table V). Margen, on the other hand, reports much higher values for the exchangable albumin pools.[21] Analysis of these data by Goldsworthy suggests that for various theoretic reasons Sterling's figures are more probably correct.[9]

Other investigators employing the radioalbumin technique have reported albumin half-lives for normal subjects within the range of values observed in this study (Table VII). One notable exception was Berson et al[2] who noted a relatively longer half-life (17.0 days). These investigators postulated that the distribution of the iodoalbumin does not occur completely for 4 to 7 days after administration. Consequently, inclusion of these points in plotting the degradation curves was felt to contribute to erroneously faster turnover rates. In the present study, no appreciable difference in turnover rate could be detected by plotting the degradation curves on points following the 7th day as opposed to using the entire exponential portion of the curve. Sterling and Steinfeld[29] have pointed out

that Berson's "normal" data were obtained from patients with chronic disorders, but with normal serum albumin levels. Steinfeld has demonstrated that the half-life in such patients is several days longer than in truly normal subjects.[29]

The various theoretic aspects of the radioiodinated albumin technique which are discussed above are of significance in terms of the absolute values of albumin metabolism. However, since there exists a direct relationship between the radioiodinated albumin and the biosynthetic isotope method, which is presumably more reliable, the use of the former method is valid in studying relative variations in albumin metabolism in various diseases.

It is surprising that the mean albumin turnover rate in patients with viral hepatitis was not abnormal. It seems obvious that in the average mild case of the disease, albumin metabolism does not differ substantially from the normal state. However, in the most severely ill patients, in whom one would expect the greatest abberations in albumin turnover, the use of adrenocortical steroids obscured the validity of the observations, and consequently such possible abnormalities. Further investigation of patients with severe hepatitis uncomplicated by adrenocortical steroid therapy is needed.

Summary:

Radiodinated albumin was used to study the albumin metabolism of 10 patients with viral hepatitis and in normal and cirrhotic control subjects. There was no significant abnormality in albumin metabolism in the group of hepatitis patients, although the albumin half-life appeared to be related inversely to the severity of the hepatitis. Three patients studied before and after recovery from the acute phase of their illness showed no significant change in albumin metabolism. Two patients treated with adrenocortical steroids had accelerated albumin turnover rates, which confirmed well established effects of such preparations on albumin metabolism. The many factors which affect albumin metabolism in this and similar studies as measured by various isotopic techniques were related to artifacts of synthesis and variations in procedure.

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Appendix:

Clinical Summaries:

A summary of the maximal values for liver function tests of the patients with hepatitis is found in Table IX.

R.H.; One month prior to admission, had gradual onset of nausea, malaise, fever, chills, dark urine, light stools and icterus. Wife also icteric; physical examination revealed yellow sclerae, tender liver palpable 2 fbs below costal margin; hospital course restricted to bed rest and supportive therapy, discharged 2 weeks after admission with normal liver function tests. (29 y.o. white, married male)

R.F.; 33 y.o. white married male; two week history of malaise, fatigability, myalgia, headaches, chills, light stools, dark urine, jaundice; afebrile; physical examination revealed slight icterus, liver down 3 fbs, markedly tender. Treated supportively with bed rest, discharged asymptomatic and with normal liver function tests three weeks after admission.

J.D.; Tubercular and alcoholic patient, 52 y.o. white male, was being maintained on PZA when malaise, anorexia, dark urine and jaundice appeared with an increase in B.S.P. retention and rise in SGOT. There was marked hepatomegaly, 3-4 fbs below costal margin, and palmar erythema. Persistence of symptoms led to liver biopsy, which revealed severe hepatitis with acute necrosis. Treatment was supportive, and patient's liver function gradually returned to normal, however, hospitalization continued because of pulmonary problem.

E.A.; 37 y.o. white male with 1 week history of sore throat, fever, was given penicillin with subsequent swelling of hands, blistering and swelling of feet and legs, aching, and generalized pruritis. Liver was tender, enlarged 2 fbs below costal margin. Liver biopsy consistent with toxic hepatitis; hospital course restricted to supportive therapy, marked by gradual return to normal liver function.

E.B.; 29 y.o. negro male with 8 day history of anorexia, vomiting, fatigue, myalgia, scleral icterus, dark urine, light stools; sister had hepatitis one month prior; physical examination revealed scleral icterus, tender liver edge palpable just below costal margin; liver function tests gradually returned to normal on supportive therapy; discharged 2 weeks after admission.

Table IX

MAXIMAL VALUES FOR LIVER FUNCTION TESTS OF PATIENTS WITH HEPATITIS

Patient	Total Bili. mg%	B.S.P. % ret.	Transaminase units	Alk. Phosphatase units	Thymol Turbidity units	Cephalin Flocculation
R.H.	3.5	42.0%	72	4.8	8.6	1+/2+
R.F.	2.0	13.8%	66	5.7	12.0	2+/3+
J.D.	24.0	64.0%	830	10.3	6.8	2+/3+
E.A.	3.0	23.5%	91	20.0	1.6	0/0
E.B.	11.9	75.0%	139	7.0	13.5	3+/4+
E.M.	8.9	63.9%	244	14.6	13.8	3+/4+
R.M.	1.2	49.5%	225	27.4	6.4	1+/2+
W.E.B.	20.1	54.4%	750	9.3	13.4	3+/4+
A.D.	38.8	-	4000	10.o	11.5	3+/4+
W.B.	21.8	74.0%	125	5.0	15.0	0/0

E.M.; 29 y.o. negro male, had gradual onset on epigastric pain, with dark urine and scleral icterus 6 days prior to admission; 4 out of 6 members of family had recently had hepatitis; no hepatomegaly; liver biopsy revealed viral hepatitis; treated supportively, with rapid return to normal liver function.

R.M.; 28 y.o. negro male; five days prior to admission had nausea, vomiting, dark urine; physical examination revealed minimal right upper quadrant tenderness, tender, round liver edge 2 fbs below costal margin, no icterus. Hospital course supportive; discharged three weeks after admission with normal liver function.

A.D. 64 y.o. white male; two months prior to admission, had hemorrhagic episode from peptic ulcer requiring transfusion with 2 units blood; two weeks prior to admission, noted onset of anorexia, weakness, malaise, vomiting, dark urine, light stools, scleral icterus; had 15-20 lb. weight loss. Physical examination revealed marked icterus, bilateral palmar erythema, suggestion of fullness of liver edge. Total bilirubin continued to rise, prednisone and then ACTH therapy instituted. Liver biopsy revealed severe viral hepatitis with subacute hepatic necrosis. Liver function tests began to gradually improve, then suddenly had severe, sharp epigastric pain with guarding and peritoneal rebound tenderness- perforated duodenal ulcer - developed ileus- treated with nasogastric suction, i.v. fluids, antibiotics, pressors - patient recovered, but had another acute episode some days later, was unresponsive to therapy, and expired.

W.E.B.; 35 y.o. white male had 1 week history of nausea and vomiting and one day prior to admission noted dark urine and scleral icterus. Physical examination revealed severe jaundice, but no organomegaly with only mild epigastric distress' Liver biopsy revealed subsiding viral hepatitis; had asymptomatic hospital course, received supportive therapy, discharged 5 weeks after admission.

W.B.: 35 y.o. negro male; noted myalgias and arthralgias 3 weeks prior to admission.Gradual appearance of icteric sclerae, pruritis, dark urine, gray stool; physical examination revealed scleral and palatine icterus, tender liver 3 fbs below costal margin. Serum bilirubin continued to climb, liver biopsy revealed severe infectious hepatitis; clinical symptoms became more severe, patient begun on prednisone and then ACTH therapy with rapid alleviation of symptomatology and return to normal of hepatic function. Discharged asymptomatic 4 weeks after admission.

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